

Molecular cloning of a novel protein-tyrosine phosphatase SH-PTP3 with sequence similarity to the *src*-homology region 2*

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Protein-tyrosine phosphorylation and dephosphorylation are directly associated with cellular growth, signal transduction, and neoplastic transformation. Here we report the isolation of a complementary DNA (cDNA) clone encoding a novel protein-tyrosine phosphatase (PTP) from a human T cell PEER cDNA library. The predicted open reading frame encodes a ~68-kDa protein composed of 593 amino acids which contains two *src*-homology region 2's (SH2 domains) at the N terminus; this PTP is designated as SH-PTP3. Northern blot analysis revealed that SH-PTP3 mRNA was expressed throughout many tissues and the transcriptional size was consistent at about 6.0 kb. As with other SH2 domains in *src*-family kinases, the SH2 domains of SH-PTP3 may play a crucial role in interactions with tyrosine phosphorylated signaling proteins, including itself and protein tyrosine kinases (PTKs), to regulate targets' enzyme activity.

Protein-tyrosine phosphatase; Protein tyrosine kinase; *src*-Homology region 2

1. INTRODUCTION

A variety of growth factors can lead protein tyrosine phosphorylation through receptor-linked protein-tyrosine kinases (PTK) or their associated PTKs [1,2]. The signals are mostly initiated by autophosphorylation of the receptors [3] and subsequently transduced via intracellular signaling pathways. In these signalings, *src* homology region 2 (SH2) has a key role in the signaling process because tyrosine autophosphorylation of the receptors result in binding sites for proteins with SH2 regions [4], for example, phospholipase C- γ (PLC- γ), and GTPase-activating protein (GAP) [5,6].

During signal transduction, phosphorylated tyrosines within the regulatory domains in *src*-family kinases can be specifically dephosphorylated with corresponding protein-tyrosine phosphatases (PTP) so as, ultimately, to increase signal transduction; this has been demonstrated for interactions between CD45 and p56^{lck} [7] or p59^{fyn} [8]. Thus, cross-talk among PTKs and PTPs is likely to have a crucial role in the control of cellular growth and differentiation. Recently, a PTP (PTP1C [9], SH-PTP1 [10] or HCP [11]) which contains SH2 do-

main has been identified and demonstrated to form high-affinity complexes with the tyrosinephosphorylated epidermal growth factor receptor [9]. Although the biological functions of SH-PTP1 remain to be elucidated, the PTP may directly participate in signal transduction pathways.

We have previously identified several PTPs expressed in pre-B cell NALM-6 by the reverse transcriptase-polymerase chain reaction (RT-PCR) technique [12], and have characterized a non-transmembrane PTP gene, LC-PTP [13] or HePTP [14], by the use of the PCR cDNA probe (BPTP-4) [12]. Here, we report the full-length cDNA sequence of another novel non-transmembrane PTP (SH-PTP3), which has been cloned using another cDNA probe (BPTP-3) [12]. SH-PTP3 possesses two SH2 domains at the amino (N)-terminus and shows a strong homology with SH-PTP1, but a fairly different nucleotide sequence. Thus, our data provide another SH2-encoding PTP, supporting the earlier suggestion of complicated cross-talk between PTKs and PTPs.

2. MATERIALS AND METHODS

2.1. Isolation of LC-PTP cDNA clones

A PEER cDNA library (Clontech) was probed with a 0.26-kb BPTP-3 PCR product which had been isolated from total RNA of NALM-6 cells by RT-PCR [12]. Approximately 1.2 million plaques were transferred to nitrocellulose filters (Millipore) and screened. The hybridization conditions were 50% formamide/2 \times SSC (1 \times SSC is 150 mM NaCl/15 mM trisodium citrate)/2.5 \times Denhardt's solution, 0.1% SDS, 2.5% dextran sulfate, and denatured calf thymus DNA (100 μ g/ml). The filters were washed with 2 \times SSC at 50°C and exposed to Amersham hyperfilms using an intensifying screen. Hybridizing

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Abbreviations: PTP, protein-tyrosine phosphatase; PTK, protein tyrosine kinase; PCR, polymerase chain reaction; cDNA, complementary DNA.

*The sequence reported in this paper have been deposited in the EMBL/GenBank data base under the accession number D13540.

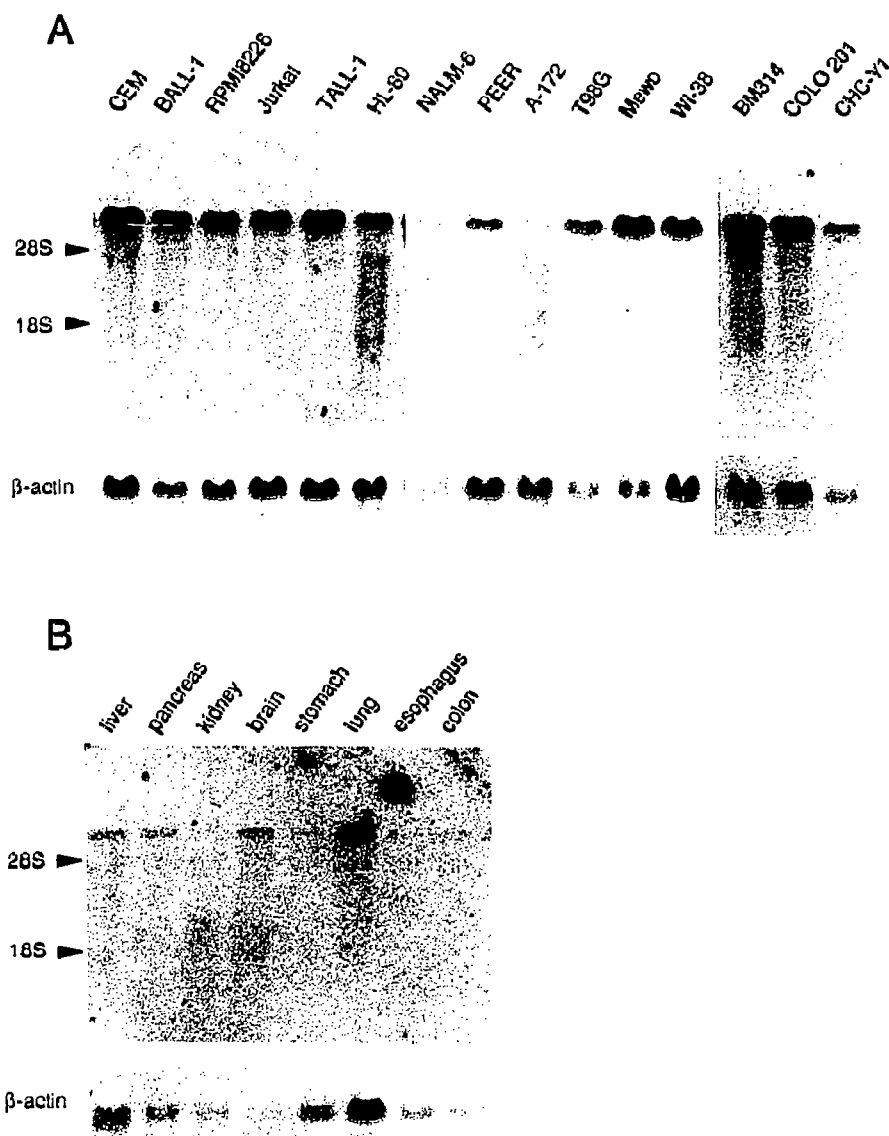


Fig. 2. RNA transfer blot analysis of SH-PTP3 transcript. (A) Total RNA samples were extracted from human T cell leukemia lines (CEM, Jurkat, TALL-1, PEER), B cell malignant cells (BALL-1, RPMI8226, NALM-6), myeloid cell lines (HL60), glioblastoma (A-172, T98G), malignant melanoma (Mewo), pulmonary fibroblast (WI-38), and colon carcinoma cells (BM314, COLO 201, CHC-Y1), and (B) from human tissues from an autopsy subject. Ten μ g of total RNAs were electrophoresed (1% agarose gel), transferred to nitrocellulose filters, and hybridized with the 32 P-labelled SH-PTP3 cDNA probe. After the first autoradiography, the same filters were rehybridized with 32 P-labeled β -actin cDNA probe to confirm equal loading (bottom). The positions of ribosomal RNA markers are indicated to the left of the blots.

striking feature of SH-PTP3 is that this contains two SH2 domains at the N-terminus (Fig. 1). It is noteworthy that the SH2 domains contain the invariant residues found in SH2 domains of other molecules PTK, PLC- γ and GAP. In addition, there are two FLVRESSES motifs which have been suggested to be involved in phosphotyrosine binding [18], FLVRESQS (a.a. 135 to 142) and FLARPSKS (a.a. 29 to 36) segments (Fig. 1). Interestingly, carboxyl (C) terminus contains nuclear localization-like motif [19], KRKRK (a.a. 534 to 538) and the prolin-rich domain (a.a. 551 to 568). Furthermore, there

is a consensus sequence for ATP binding site (GXXXXGKS) [20] within the N-terminal SH2 domain (a.a. 158 to 165).

3.2. Broad expression of SH-PTP3 transcripts

Northern blot analysis of SH-PTP3 mRNA revealed approximately 6.0-kb transcripts. Initial studies of some cell lines showed SH-PTP3 transcripts in all the lines examined. A wide variety of cell lines and human tissues were then analyzed; SH-PTP3 was found in every kind of tissue we examined, though at considerably differing

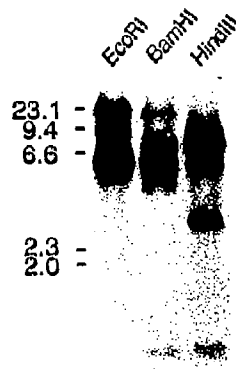


Fig. 3. Southern blot analysis of SH-PTP3. Ten μ g of genomic DNA from MOLT-3 was digested with the indicated restriction enzymes, electrophoresed (0.7% agarose gel), transferred to nitrocellulose filters, and hybridized with the 32 P-labelled SH-PTP3 cDNA probe. Mobility of DNA standards (kb) is shown at left.

levels of expression (Fig. 2). It was present at somewhat high levels in our one sample of brain tissue (Fig. 2B).

3.3. Southern blot analysis of SH-PTP3

Southern blot of human genomic DNA was hybridized with SH-PTP3 cDNA probe containing the catalytic domain. The blots revealed several bands of hybridization (Fig. 3), indicating that either the gene is huge with many introns or that there are multiple genes for SH-PTPs. Since the Northern blot analysis revealed a single transcript (~ 6.0 kb), it is likely that the SH-PTP3 gene is very large and consists of complicated exon-intron organizations. These multiple bands constantly appeared in at least three independent experiments with high stringency (data not shown).

4. DISCUSSION

We have isolated a novel non-transmembrane PTP (SH-PTP3) with two SH2 domains from a human T cell leukemia cell PEER cDNA library. Recently, a PTP with two SH2 domains has been identified in many laboratories [9–11]; it has been dubbed with a correspondingly wide variety of names, but because of some striking features of its structure and preliminary data suggesting additional SH2-encoding PTPs, the name SH-PTP1 [10] is used in this presentation. Overall, our novel SH-PTP3 is highly homologous to the SH-PTP1 (71% identity). Nucleotide sequence similarity in the coding regions is not so high (39.6% identity), which appears to have been the reason we could not detect transcripts of SH-PTP1 (2.6 kb) with SH-PTP3 probes

SH-PTP3	MTSRKHFHM ITGVEAENLLLTGCGGSLAPRPRSRHPDPTLVRNRGAVTHIKIQTNGDYD
SH-PTP1	NV R HFHNDLGLDAETLAKGAVIGSFLAPRPRSRHPDPTLVRNRGAVTHIRIQNGDYD
SH-PTP3	YGGKVFATLALVQYTYNHHQKREKNDVIELKYPINCAPTSERMFIHGLSGKEAKLLETKGNI
SH-PTP1	TGGKVFATLALVQYTYNHHQKREKNDVIELKYPINCAPTSERMFIHGLSGKEAKLLETKGNI
SH-PTP3	GSEFLVRESGHPGDFVLVRHGDDKESHDGKRVTHVNRGQELKTDVCGSERPDLTDLVEHY
SH-PTP1	HTFLVRESLSPGDFVLVR LSGPKAGDGPFLVRTHVNRGEGRYTVCGLTDFDLTDLVEHY
SH-PTP3	KKHDMATLCTVLLQKQPLHTTINAAHIESRVSLKLAETTKVKQGVHSEFTLQGGKLLYS
SH-PTP1	KKTCLEADGAFVYLRQYATVWNAOTENRVLELHKQGLSEDTAKAGQWEEPELAKSEKNNLH
SH-PTP3	RKEGQGRWIKIRNRKNTLFPQTRVVLHGGDNEPVDYINANI THNHTTKKNSHKPKKVIATQ
SH-PTP1	RLEGRPEREKRRNTLFPQTRVVLHGGDNEPVDYINANI THNHTTKKNSHKPKKVIATQ
SH-PTP3	GCLQNTVNDPFWHMFQSENRVIVMTTKEVERGSKCKVNYGDEYALKEVGVSRVRVRESANIDYTL
SH-PTP1	GCLDAIVNDPFWHMFQSENRVIVMTTKEVERGSKCKVNYGDEYALKEVGVSRVRVRESANIDYTL
SH-PTP3	RELKL SKVGQGRTERVHQYHRTWPDHGVSDPQGVDFLSEVHKKQESIMDAGVWVHCS
SH-PTP1	ASYKLRTAQLSELDNGDLVREHVIQYLSHPDHGVSDPQGVDFLSEVHKKQESIMDAGVWVHCS
SH-PTP3	AGIGKGTFLVIDILIDIIREKGVDCIDVPTIQMVRSGHGMQTEAGTYFTYNAVQIYIETLGR
SH-PTP1	AGIGKGTFLVIDILIDIISTKGLDCIDVPTIQMVRSGHGMQTEAGTYFTYNAVQIYIETLGR
SH-PTP3	RIEESGRKAKKHETTHINYPADQTSQGGSPAPCTPTPCANRDEGSAVYERGLM
SH-PTP1	KLAVLQGGQGGSEYGHITTPAKNNHIA KASIVSS HUKED VYENHNTKGRSEKVK
SH-PTP3	QGGKSR
SH-PTP1	KQSDAKENSGKSLKSK

Fig. 4. Amino acid sequence comparison between SH-PTP3 (rat and human) and SH-PTP1. Two FLVRES motifs were shown by bold-face type. Amino acid sequence available for rat SH-PTP3 (L1) is overlined and the arginine (R) indicates an amino acid which is different from human SH-PTP3 sequence. The putative ATP binding domain is overlined with a broken line and the consensus sequence for nuclear localization signal is double overlined. The proline-rich domain is indicated by asterisks. Gaps (space) were introduced to optimize alignments.

under our conditions. Interestingly, SH-PTP3 is expressed throughout many tissues (Fig. 2), whereas SH-PTP1 is predominantly expressed in hematopoietic cells [11]. These different expression patterns may be associated with their biological functions.

The evidence summarized above conclusively demonstrates, we believe, that there is a subfamily of PTPs with SH2 domains. SH2 domains contain approximately 100 amino acids that are highly conserved among the *src* family members, and recently have also been found in cytoplasmic signaling proteins including PLC- γ , GAP, an 85-kDa subunit of PI3'-kinase [21], and the *v-crk* oncogene [22]. The roles of the SH2 domains of these signaling proteins suggest several possible functions for the SH2 domains in SH-PTP3. First, as the SH2 domains of *src*-like kinases [23], SH2 domains of SH-PTP3 may interact with its own tyrosine phosphorylation sites to negatively regulate enzyme activity. It is necessary to investigate whether SH-PTP3 itself can be tyrosine phosphorylated. Second, SH2 domains directly interact with signaling proteins and the resultant complexes may activate them or make them accessible to specific substrates by tyrosine dephosphorylation. Third, SH2 domains can target activated signaling proteins for signal termination by reversing tyrosine phosphorylation.

Recently, PTP L1 has been purified from rat liver cytosol; the amino acid sequence of the peptides indi-

cates that PTP L1 [24] has extensive similarity (only one a.a. difference within the available sequence) to SH-PTP3 (Fig. 4), implying that they are species homologs. The molecular mass of purified L1 is estimated to be 67 kDa by SDS-PAGE [24], which is similar to the molecular weight (68 kDa) of the predicted protein for SH-PTP3. Interestingly, L1 preferentially dephosphorylates tubulin [24], suggesting L1 (SH-PTP3) can be associated with microtubule-based motility. In view of the nucleotide-dependent interaction of SH-PTP3 with microtubules, it is of interest that SH-PTP3 possesses the consensus sequence for ATP binding, GXXXXGKS [20], which is found in SH-PTP3 sequence beginning at Gly¹⁵⁸.

Interestingly, SH-PTP3 has a nuclear localization signal-like segment. So far two PTKs (*c-abl* and *c-fer*) have been localized to the nucleus [25,26]. Their biological functions are being studied extensively, but are still obscure. Recently, loss of phosphotyrosine in p34^{cdc2} was shown to be associated with activation of intrinsic serine kinase activity and appears to be a necessary step in the G2 to M transition in cell cycle [27]. Importantly, p35^{cdc25} has been demonstrated to manifest intrinsic PTP activity; it dephosphorylates the phosphotyrosine of p34^{cdc2} [28]. Thus, the enzyme activity of some serine/threonine kinases is also likely to be regulated by their own tyrosine-phosphorylation and dephosphorylation, suggesting important roles of SH2 domains. SH-PTP3 is ubiquitously expressed in human tissues and possesses a nuclear localization signal. These data suggest the attractive prospect that SH-PTP3 can participate in the modification of phosphotyrosine, a crucial step in the cell cycle.

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REFERENCES

- [1] Hunter, T. and Cooper, J.A. (1985) *Annu. Rev. Biochem.* 54, 879-930.

- [2] Yarden, Y. and Ullrich, A. (1988) *Annu. Rev. Biochem.* 57, 443-478.
- [3] Molra, A.O., Schreurs, J., Miyajima, A. and Wang, J.Y.J. (1988) *Mol. Cell Biol.* 8, 2214-2218.
- [4] Koch, C.A., Anderson, D., Moran, M.F., Ellis, C. and Pawson, T. (1991) *Science* 252, 668-674.
- [5] Kaplan, D.R., Morrison, D.K., Wong, G., McCormick, F. and Williams, L.T. (1990) *Cell* 61, 125-133.
- [6] Margolis, B., Rhee, S.G., Felder, S., Mervic, M., Lyall, R., Levitzki, A., Ullrich, A., Zilberstein, A. and Schlessinger, J. (1989) *Cell* 57, 1101-1107.
- [7] Mustelin, T., Coggeshall, M.K. and Altman, A. (1989) *Proc. Natl. Acad. Sci. USA* 86, 6302-6306.
- [8] Mustelin, T., Pessa-Morikawa, T., Autero, M., Gassmann, M., Andersson, L.C., Gahrberg, C.G. and Burn, P. (1992) *Eur. J. Immunol.* 22, 1173-1178.
- [9] Shen, S., Bastien, L., Posner, B.I. and Chretien, P. (1991) *Nature* 352, 736-739.
- [10] Plutsky, J., Neel, B.G. and Rosenberg, R.D. (1992) *Proc. Natl. Acad. Sci. USA* 86, 1123-1127.
- [11] Yi, T., Cleveland, J.L. and Ihle, J.N. (1992) *Mol. Cell Biol.* 12, 836-846.
- [12] Adachi, M., Sekiya, M., Arimura, Y., Takekawa, M., Itoh, F., Hinoda, Y., Imai, K. and Yachi, A. (1992) *Cancer Res.* 52, 737-740.
- [13] Adachi, M., Sekiya, M., Isobe, M., Kumura, M., Ogita, Z.-I., Hinoda, Y., Imai, K. and Yachi, A. (1992) *Biochem. Biophys. Res. Commun.* 186, 1607-1615.
- [14] Zanke, B., Suzuki, H., Kishihara, K., Mizzen, L., Minden, M., Pawson, A. and Mak, T.W. (1992) *Eur. J. Immunol.* 22, 235-239.
- [15] Kozak, M. (1984) *Nucleic Acids Res.* 12, 857-873.
- [16] Streuli, M., Krueger, N.X., Hall, L.R., Shlossman, S.F. and Saito, H. (1988) *J. Exp. Med.* 168, 1523-1530.
- [17] Krueger, N.X., Streuli, M. and Saito, H. (1990) *EMBO J.* 9, 3241-3252.
- [18] Mayer, B.M., Jackson, P.K., Van Etten, R.A. and Baltimore, D. (1992) *Mol. Cell Biol.* 12, 609-618.
- [19] Landford, R.E. and Butel, J.S. (1984) *Cell* 37, 801-813.
- [20] Walker, J.E., Sarasata, M., Runswick, M.J. and Gay, N.J. (1982) *EMBO J.* 1, 945-951.
- [21] Otso, M., Hiles, I., Gout, I., Fry, M.J., Ruiz-Larrea, F., Panayotou, G., Thompson, A., Dhand, R., Hsuan, J., Totty, N., Smith, A.D., Morgan, S.J., Courtneidge, S.A., Parker, P.J. and Waterfield, M.D. (1990) *Cell* 65, 91-104.
- [22] Cantley, L.C., Auger, K.R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. and Soltoff, S. (1991) *Cell* 64, 281-302.
- [23] Cooper, J.A., Gould, K.L., Cartwright, C.A. and Hunter, T. (1986) *Science* 231, 1431-1434.
- [24] Hiraga, A., Munakawa, H., Hata, K., Suzuki, Y. and Tsuiji, S. (1992) *Eur. J. Biochem.* (in press).
- [25] Van Etten, R.A., Jackson, P. and Baltimore, D. (1989) *Cell* 58, 669-678.
- [26] Hao, Q.-L., Ferris, D.K., White, G., Heisterkamp, N. and Groffen, J. (1991) *Mol. Cell Biol.* 11, 1180-1183.
- [27] Lewin, B. (1990) *Cell* 61, 743-752.
- [28] Dunphy, W.G. and Kumagai, A. (1991) *Cell* 67, 189-196.